*Reprinted from Pakistan Journal of Science, Vol. 21, No. 1 and 2, Jan -March 1969*

# A Normal Table of *Rana tigrina* Daudin

## 1. EARLY DEVELOPMENT (Stages 1-27)

M. S. KHAN,
*Department of Zoology,*
*T. I. College, Rabwah.*

Advances in the Experimental Embryology have necessitated a system of arrangement of various stages during the normal development of an animal, in a systematic chronological order. These stages must be readily recognizable and easy to detect. Since twentieth century many workers have worked out the chronological tables of various animals i.e. fishes, amphibians, reptiles, birds and mammals.

**Fishes**

*Clupea pallasi* (Outram, 1957) *Oryzias latipes* (Rugh, 1965) *Xiphophorus* (Platypoecilus) *Maculatus* (Tavalga & Rugh, 1947) *Fundulus heteroclitus* (Solberg, 1938, Oppenheimer, 1936) *Solmo irideus* (Witschi, 1956) *Lepidosiren paradoxa* and *Protopterus armectens* (Kerr, 1909).

**Urodeles**

*Triturus punctatus* (Glasner, 1925) *Triturus pyrrhogaster* (Anderson, 1943) *Triturus vulgaris* (Glasner, 1925) *Triturus torosus* (Twitty and Bodenstin in Rugh, 1965) *Amblystoma punctatus* (Harrison, unpublished; Leavitt in Rugh, 1965) *Ambystoma maxicanus* (Witschi, 1956) *Necturus maculatus* (Eycleshymer & Wilson, 1910).

**Anura**

*Rana sylvatica* (Pollisteer & Moore, 1937) *Rana pipiens* (Shumway, 1940; Miller, 1939; Taylor & Kollros, 1946) *Rana fusca* (Kopsch, 1953); *Rana japonica* (Tahara, 1959) *Rana dalmatina* (Cambar & Marrot, 1954) *Xenopus leavis* (Weisz, 1945; Nieuwkoop & Faber, 1956) *Alytes obstetricans* (Cambar & Martin, 1956) *Hyla regilla* (Eakin, 1947) *Discoglossus pictus* (Gallien & Houillon, 1951) *Bufo arenarum* (del Conte & Sirlin, 1952) *Bufo bufo* (Cambar & Gipouloux, 1956; Rossi, 1958) *Bufo regularis* (Sedra & Michael, 1961) *Bufo melanostictus* (Khan, 1965) *Bufo vulgaris* (Ekin, 1946; Okada & Baba, 1932) *Bufo valliceps* (Limbaugh & Volpe, 1957).

**Birds**

Chick (Lillie, 1952; Hamilton & Hamburger, 1951).

**Mammals**

*Homosapiens* (Streeter, 1942-51; Witschi, 1962 b) Rat (Witschi, 1962 b).

Present study has been undertaken to compile the chronological table of *Rana tigrina* Daudin, upto 27th stage (Tadpole larva). Nasir-ud-Din (unpublished) has also compiled a normal table for this animal. During his study, he has failed to recognize certain very important features, as the grey crescent, the way and nature of third cleavage. In post-larval development he has used the larvae of *Rana cyanophylictus* for the larvae of *Rana tigrina*. Moreover, his findings so far have remained unpublished.

### MATERIAL AND METHOD

Material for the present study was collected, fixed and stored as reported earlier (Khan, 1965) for similar study on *Bufo melanostictus*.

During the development of the present material under study, room temperature

fluctuated between 29-32°C. Since there was no technique available for maintaining a constant temperature, it has been considered ambiguous to record the age of each stage.

## CHOICE OF STAGES

Conventionally, the amphibian development is divided in chronologically arranged series of stages, depicted in Arabic numbers. More recently, Witschi (1953, 1962b) has purposed to divide amphibian normal development into twelve phases (periods). He includes stages 1 to 6 in phase "Cleavage"; stage 7, "Blastula"; stages 8-11 "Gastrula" and so on. Cambar & Martin (1959) and Cambar & Gipouloux (1956) while following Witschi's suggestions have further numbered these phases as periods, thus phase "Cleavage" is depicted by them as $1_{1-5}$ (Segmentation) phase "Blastula" & phase "Gastrula" as $11_{1-11}$ (Morphogenetic movements) and so on. The numbering and independent division of these phases gives an impression of discontinuity. While the development is a continuous process, one change (stage) leading to the next. Therefore, the discontinuous numbering of the phases makes the matters ambiguous. Moreover the numbering of these phases do not add to clarity and make the matters more abstract and complicated. The primary motive of a normal table is to give a ready and clear picture of a definitive developmental stage. Present author believes, the over. systematisation of the staging system will lead to ambiguity and chaos.

During the present study, the conventional staging system has been followed as for *Bufo melanostictus* (Khan, 1965) and the development has been divided into phases; though this distinction is not hard and fast.

## NORMAL TABLE

| Phase | No. of Stage | Fig. No. | Diameter/ Length | Characteristics |
|---|---|---|---|---|
| Unfertilized egg | 1 | 1 | 1.2 mm | Animal hemisphere dark brown and flat showing fovea at the animal pole. |
| Fertilized egg | 2 | 2, 3 | 1.2 mm | Pigment extends up to almost 3/4 of the animal hemisphere. Grey crescent. Fertilization membrane. |
| Segmentation & Blastulation | 3 | 4 | 1.2 mm | First cleavage, vertical, passing through the animal pole dividing grey crescent into equal lateral halves. Cleavage furrow extends upto 3/4 of vegetal hemisphere. |
| | 4 | 5, 6 | 1.2 mm | Second cleavage, vertical, at right angles to the first one. First cleavage complete, second cleavage not reaching the vegetal hemisphere. |
| | 5 | 7, 8 | 1.2 mm | Third cleavage, vertical, arising as two independent furrows at right angle to first one along the lateral sides of second cleavage, tangentially passing outward thus cutting smaller blastomeres along the sides, not reaching the vegetal pole. Second cleavage complete, embryo 8-celled, four larger blastomeres in the center while two smaller on each side, pigment confined to the upper tips of blastomeres. |

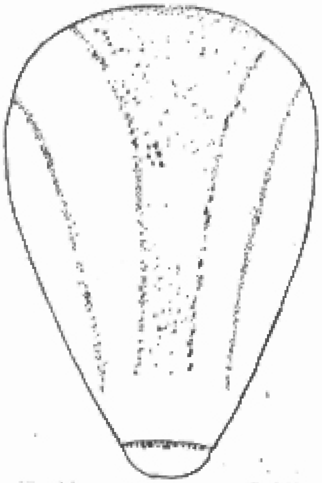

Fig.19 D.V. St.13

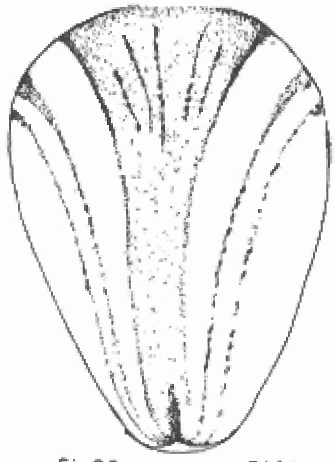

Fig20 DV. St.14

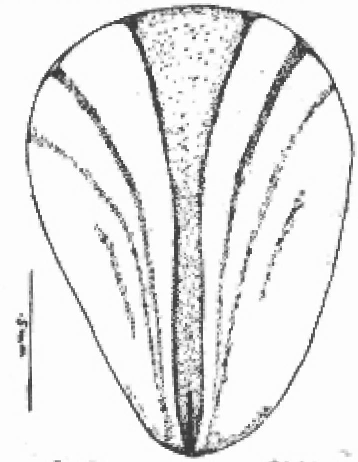

Fig21 , D.V. St.14

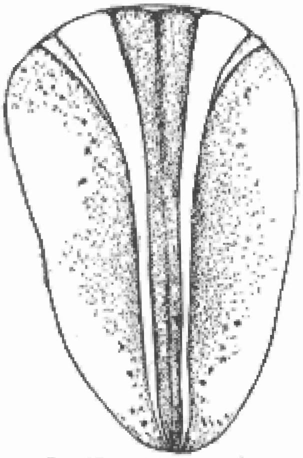

Fig22 D.V. St.15

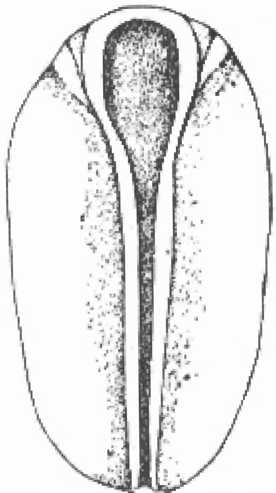

Fig23 St.15

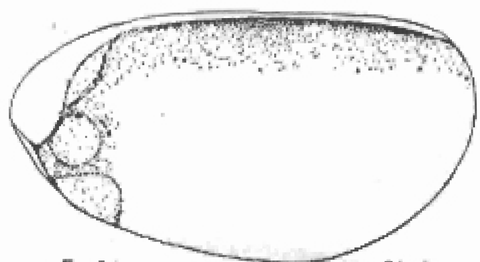

Fig24 L.V. St.15

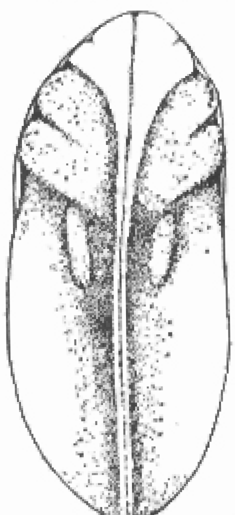

Fig25 DV. St.25

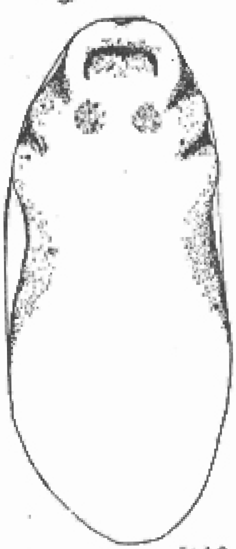

Fig.26 VV. St.16

Fig27 St.16

| | | | | |
|---|---|---|---|---|
| | 6 | 9, 10 | 1.2 mm | Fourth cleavage, horizontal, passing through tips of the blastomeres, thus cutting 8 very small dark brown micromeres, forming a horizontal layer on upper side of large megameres. |
| | 7 | 11 | 1.2 mm | Animal hemisphere roundish, Micromeres almost four time smaller than megameres and could easily be made out. Megameres of very unequal size. |
| Gastrulation | 8 | 12 | 1.2 mm | Micromeres just touching the equator, easily could not be made out. Animal hemisphere getting roundish, megameres of almost equal size. |
| | 9 | 13, 14 | 1.2 mm | Micromeres just descending the equator, girdle like flattening round the equator. Transverse aggregation of pigment along one side marks the dorsal lip of blastopore which is slit like. |
| | 10 | 15 | 1.2 mm | Crescentric blastopore |
| | 11 | 16 | 1.2 mm | Horse-shoe-shaped blastopore. |
| | 12 | 17, 18 | 1.4 mm | Round blastopores of 3/4th & 2/4th of the diameter of the embryo. |
| Neurulation | 13 | 19 | 1.8 mm | Blastopore 1/4th the diametre of the embryo, neural plate, neural folds also indicated. |
| | 14 | 20, 21 | 2.0 mm | Closure of blastopore, neural folds. |
| | 15 | 22, 23, 24 | 2.0 mm | Mid neural folds, demarcation of gill, cement gland, pronephric area. |
| | 16 | 25, 26, 27 | 2.2 mm | Closure of neural folds. Division of gill area and separation from jaw area by a groove. Formation of stomodial pit, eye area. Slight differentiation of head from main body. Pinching off of the dorsal axis of embryo. Slight indication of tail. |
| Tail Stages | 17 | 28; 29 | 2.9 mm<br>0.2 mm | Head and trunk sharply demarcated, endodermal mass sharply convex ventrally. Tail bud. Myotomes in the trunk region. Eyes quite marked. Gill area tripartite. Cement gland in the form of a pair of circular areas. Stomodial pit elongate. |
| | 18 | 30, 31 | 3.2 mm<br>0.5 mm | Embryo shows embryonic curvature along its mesial dorsal side. Stomodial pit squarish, proctodial pit quite marked leading to a tubular rectal tube. Slight movements. |
| | 19 | 32, 33 | 4.00 mm<br>0.8 mm | Hatching, spontaneous movements. Dorsal & ventral tail fins, fins opaque. Mytomes extending in tail. Stomodial pit deep and squarish. Eye cup showing choroid fissure. Second gill conical. Otic vesical and nasal pits clear. |

| | | | | |
|---|---|---|---|---|
| | 20 | 34, 35 | 4.0 mm<br>1.0 mm | First and second gill having filaments, third conical. Embryonic curvature disappears. Tail fins translucent. Quick movements of larva. |
| | 21 | 36, 37 | 4.5 mm<br>1.9 mm | Eye cup circular with the closure of choroid fissure. Tail fins less translucent. Third gill filamentous. |
| | 22 | 38, 39 | 4.8 mm<br>2.0 mm | Stomodial pit transverse. Slight indication of opercular folds. Tail fins transparent. Anal tube long. |
| | 23 | 40, 41 | 5.0 mm<br>2.4 mm | Opercular folds, third gill with filaments. Distinction between head and trunk less marked. Cornea less transparent. |
| | 24 | 42, 43 | 5.9 mm<br>2.8 mm | Full gill filaments, reaching up to the middle of trunk, mid-opercular folds. Cornea partially transparent, eye cup pigmented. Pre- & post-oral lips. Melano-phores along dorso-lateral sides of the trunk. Lateral line system. Cement glands quite apart. Nasal opening situated in a narrow deep groove-like canthus-rostralis. Rostral and caudal furrows in the endodermal mass. |
| | 25 | 44, 45 | 6.5 mm<br>3.1 mm | Opercular folds just touching the trunk. Larva more tadpole like. Thickened pre and post-oral lips. Cornea transparent. Eye cup pigmented. Melano-phores spreading on the dorsal side of base of tail. Pre-ocular part of head becoming massive. Slight displacement of eyes towards the dorso-lateral side. |
| Tadpole larva | 26 | 46, 47 | 7.8 mm<br>4.1 mm | Fused operculum, only slit like spiracle on left side, lower tips of gills-filaments showing through the spiracle. Horny beak, frilled pre and post-oral lips. Cement gland disappearing. Further accentuation in the massiveness of pre-ocular part of head. Eyes shifted to dorso-lateral side. Melanophores on dorsal side of head and tail.<br>Cloaca opening. |
| Critical stage | 27 | 48, 49 | 8.2 mm<br>4.5 mm | Spiracle circular leading into a spout. No indication of gill filament externally. Melanophores on the lateral side of tail. Corneafully transparent. Anal tube transparent, median, slightly deflected toward right. Slight indication of hind limb bud. Pre- and post-oral lips of sucker each showing one row of denticles. Cement gland indicated by pigmented spots. Movements of larva rapid and quick. Feeding takes place. |

# PECULIAR FEATURES IN THE DEVELOPMENT OF *RANA TIGRINA* DAUDIN

Fresh laid eggs of *Rana tigrina* have a flattened animal hemisphere. Pigment extends up to the equator, falling short of the 3/4th of the animal hemisphere. Vegetal hemisphere on the other hand is distinctly hemispherical and cream-coloured. This gives the egg very unequal animal and vegetal halves. The cytoplasm is confined in the fla tened half of the animal hemisphere in the form of a flat dark brown germinal disc, lying over the heavily yolked vegetal hemisphere. Nasir-ud-Din (unpublished) notes the flattening of the animal hemisphere after the fertilization. He attributes this phenomenon to the exudation of fluid from the egg after fertilization, with the subsequent collapse of animal hemisphere. Present study, however, reveals that his findings are erroneous, at the time of laying the animal hemisphere of egg is flat and apparently it has no connection with the fertilization.

Subsequent development of the egg favours the above view. The cleavage furrows behave like that of heavily yolked eggs. First cleavage as usual is vertical, before it reaches the vegetal pole the second cleavage, which is also vertical, appears. Thus the cleavage furrows behave like those of *Lepidosiren paradoxa* (Kerr, 1909) *Acipenser sturio* and *Lepidosteus osseus* (Dean, 1895) *Alytes obstetricans* (Cambar & Martin, 1959) which have heavily yolked eggs.

The lagging behind of the vegetal hemisphere in the cleavage tempo is the characteristics of heavily yolked eggs (Balinsky, 1965). Though, subsequently, lagging megameres pass through as many cleavages as the micromeres (Sirakami, 1958b, Dan, 1960).

The third cleavage is characteristic of this species. It is always horizontal in almost all the other anura and urodela so far reported. In *Rana tigrina* it is again vertical, appearing as two independent furrows at right angle to the first one, lateral to the second cleavage furrow and passing tangentially towards the equator. Thus cutting smaller lateral blastomeres. The tangential deviation of this cleavage points out the heavy yolky condition of the vegetal pole. However, the fourth cleavage is horizontal dividing the egg into sixteen cells. This also behaves very characteristically. It passes almost through the tips of the blastomeres to which the pigment is confined, thus cutting very small micromeres forming a blastoderm like layer over the large heavily yolked megameres. Third cleavage hence behaves like the fourth cleavage of other amphibia, perhaps here it takes place procociously. In *Necturus maculosus* the third cleavage is "irregularly vertical" (Nelson, 1953) thus bringing a condition close to *Rana tigrina*. Lung fish *Lepidosiren*, *Acipenser* and *Amia* (Jollie, 1962) show similar cleavage. Moreover the tail bud stage of *Rana tigrina* closely resembles with that of *Lepidosiren*. These similarities suggest that *Rana tigrina* is primitive than the other Ranids, reported so far, and is close to the ancestral lung fish like form.

Nasir-ud-Din also fails to recognize the grey crescent, though it is very clearly indicated, almost half an hour after the fertilization. The grey crescent could easily be followed to subsequent early development. However, the grey crescent is more or less colourless, and is indicated by the concentric displacement of the pigment along one side of the animal hemisphere (Fig. 2 to 10).

The process of neurulation starts earlier than reported for the other anura. At stage 13 (Fig. 19) when still the blastopore is quite large, the embryo is cranio-caudal elongated and the neural plate and the neural folds indicated, thus initiating the process of neurulation.

The disposition of embryo at the post-neurula stage, over the yolky mass resembles with that of animals with heavily yolked eggs i.e., fishes, non-aquatic anurans, urodeles and birds. Though this condition persists for comparatively smaller duration.

## ABBREVIATIONS

| | |
|---|---|
| A.V. | Animal view |
| D.V. | Dorsal view |
| L.V. | Lateral view |
| Fig. | Figure |
| St. | Stage |
| V.V. | Ventral view, Vegital view |

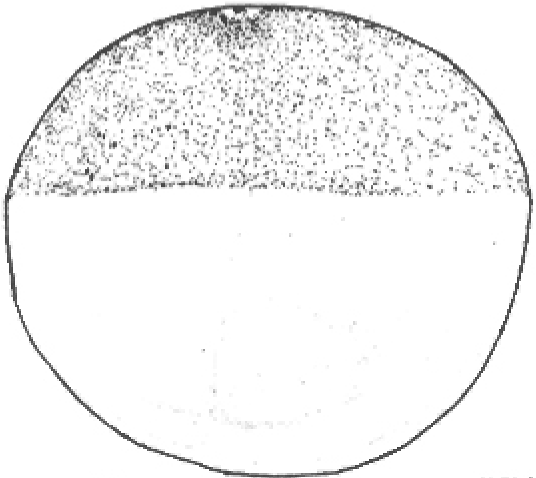

FIG.1 ST.1

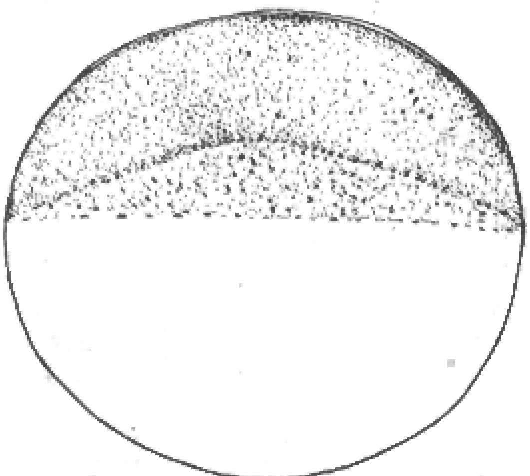

Fig.2 St.2

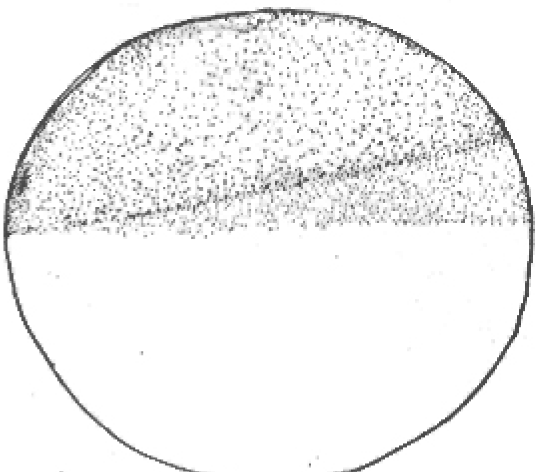

Fig 3 LV. St.2

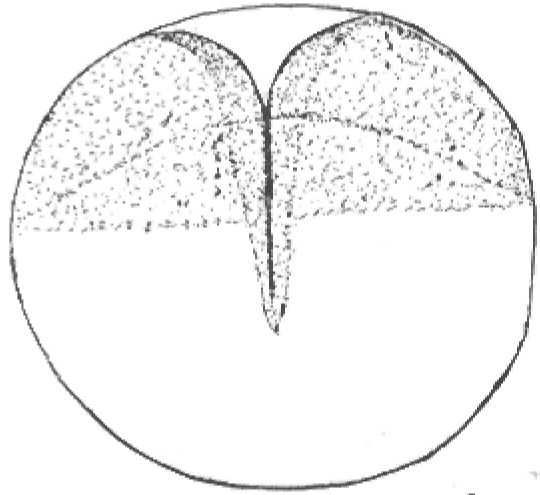

Fig.4 St.3

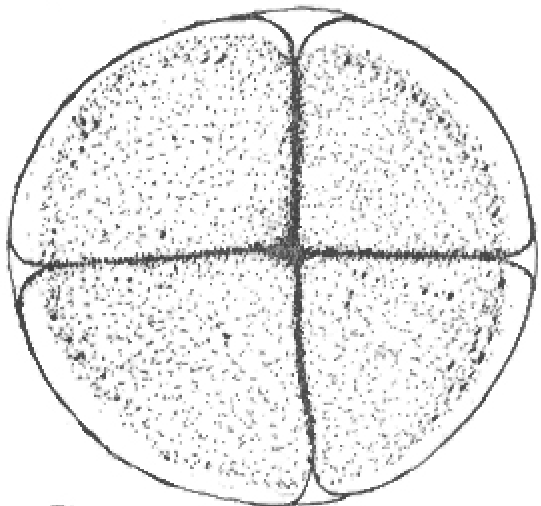

Fig.5 A.V St. 4

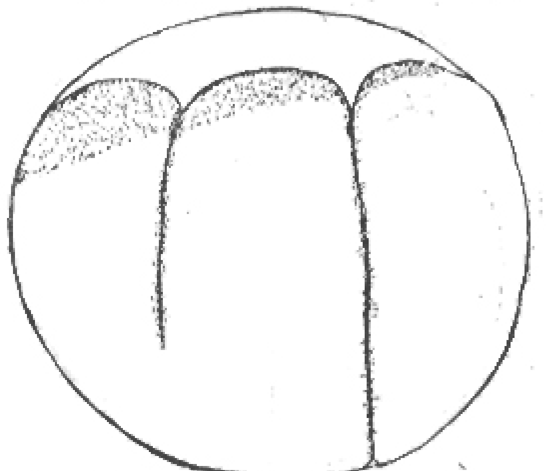

Fig.6 St 4

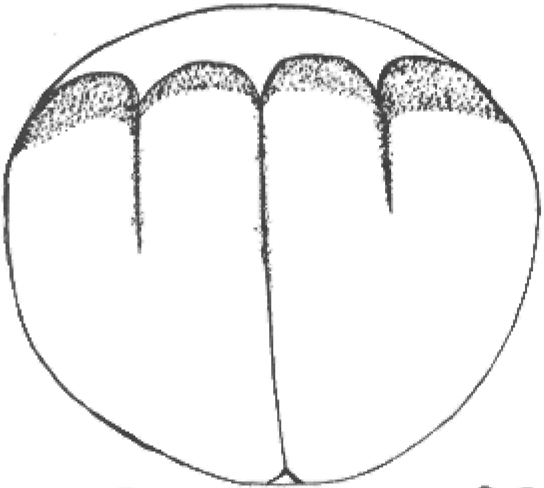

Fig.7 St.5

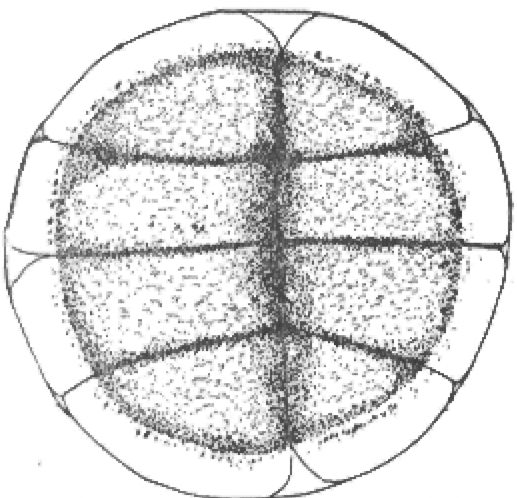

Fig.8 St.5

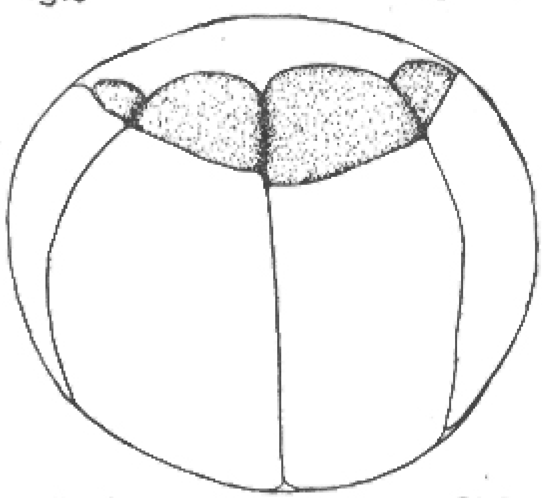

Fig.9 St.6

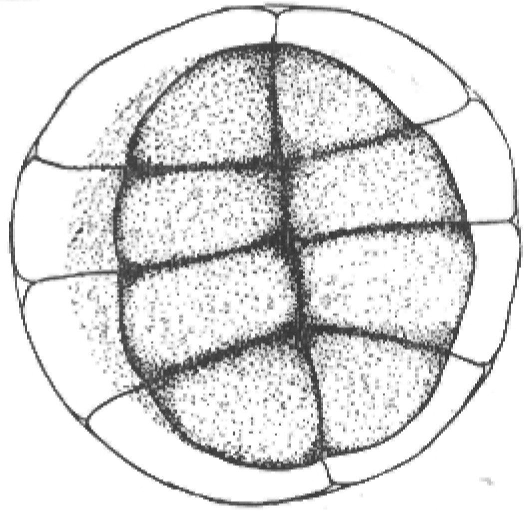

Fig.10 A.V. St.6

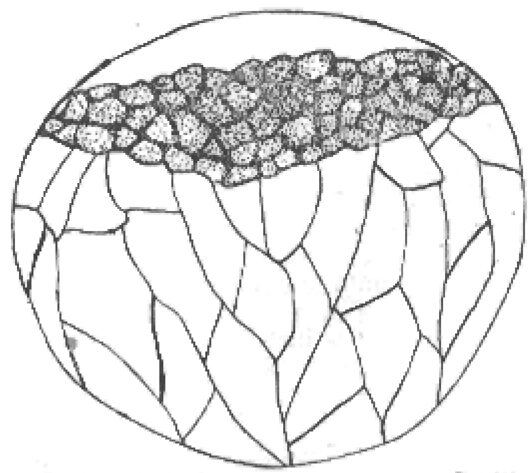

Fig.11 St.7

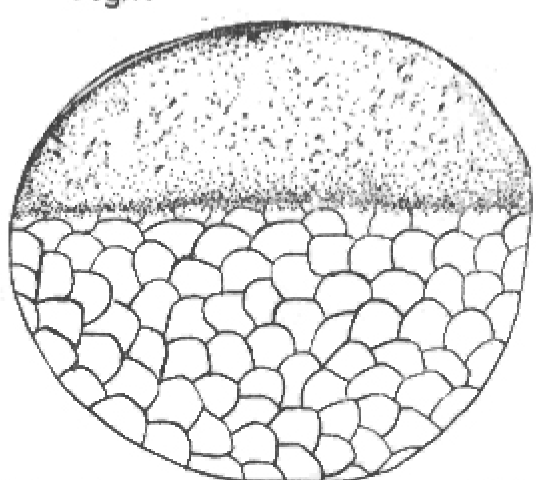

Fig.12 St8

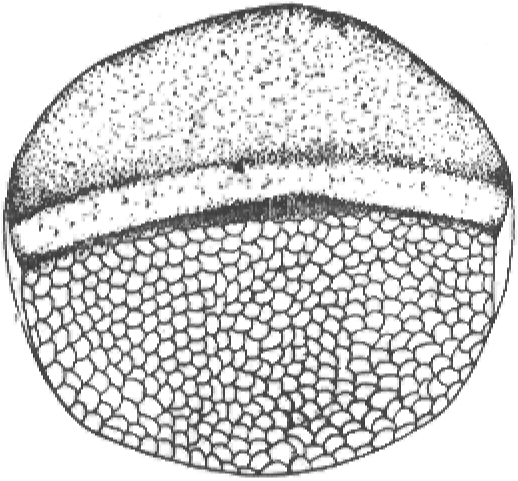

Fig.13 St9

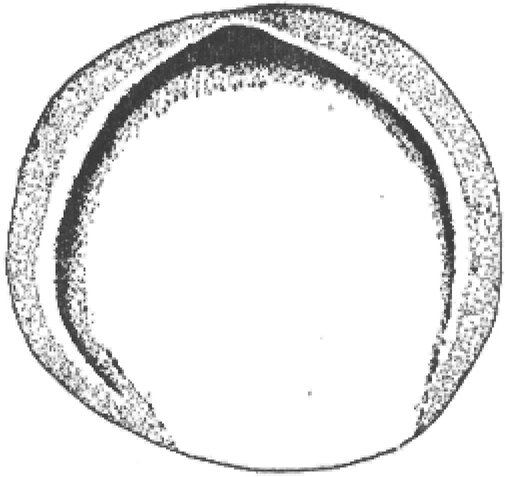

Fig.16 V.V. St.11

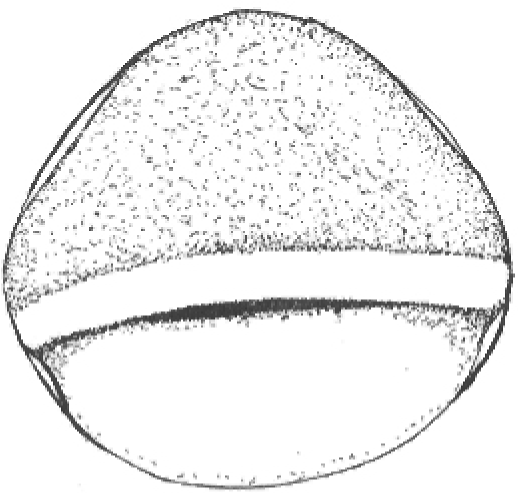

Fig.14 St.9

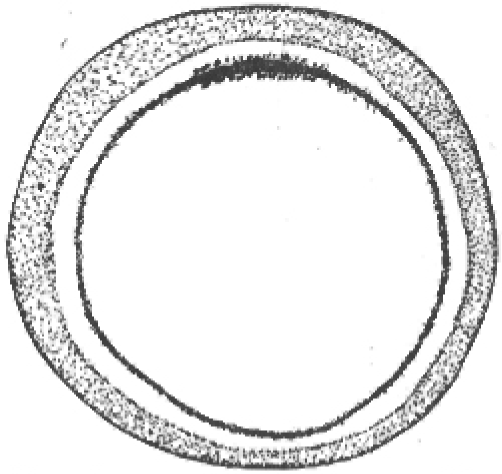

Fig 17 V.V. St.12

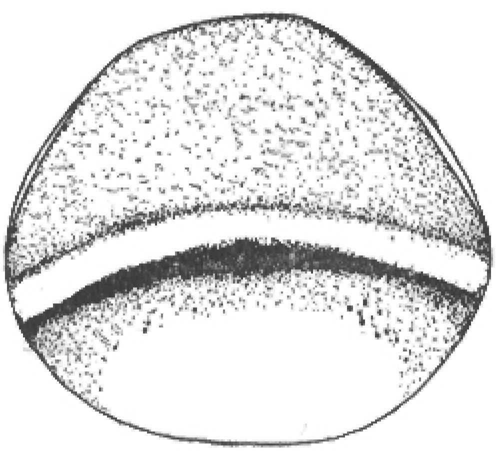

Fig.15 St.10

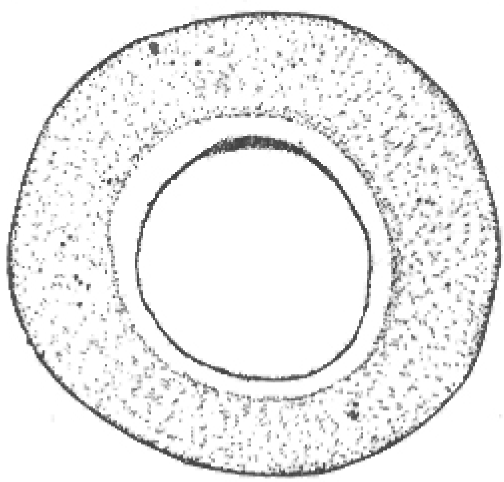

Fig.18 V.V. St.12

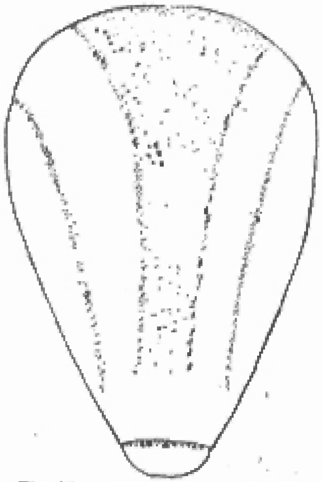
Fig.19 D.V. St.13

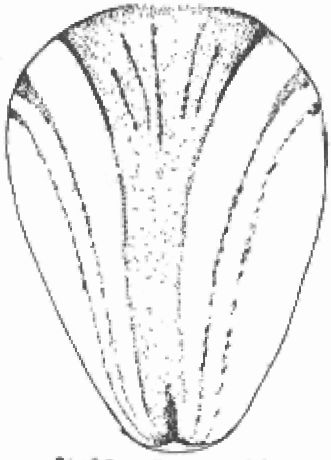
Fig20 D.V. St.14

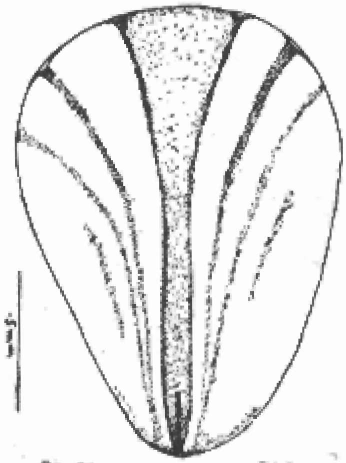
Fig21 . D.V. St.14

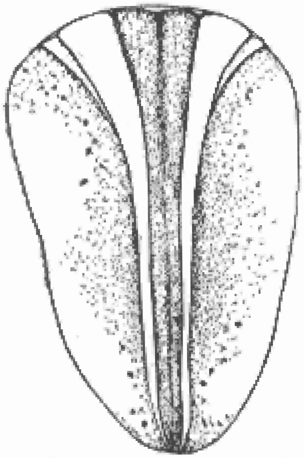
Fig22 D.V. St.15

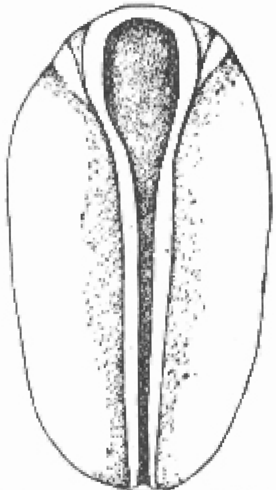
Fig23 St.15

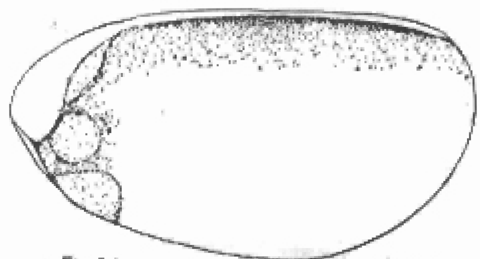
Fig24 L.V. St.15

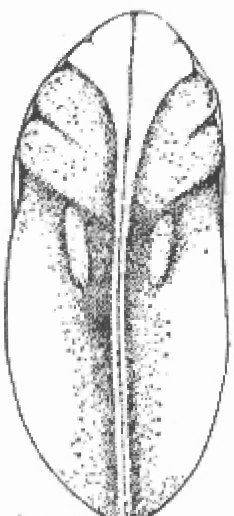
Fig25 D.V. St.25

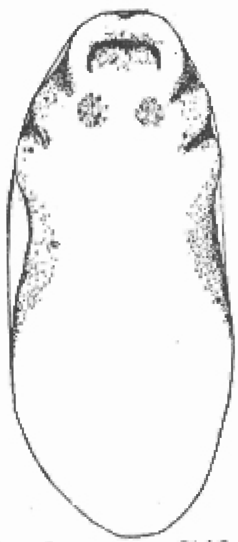
Fig26 V.V. St.16

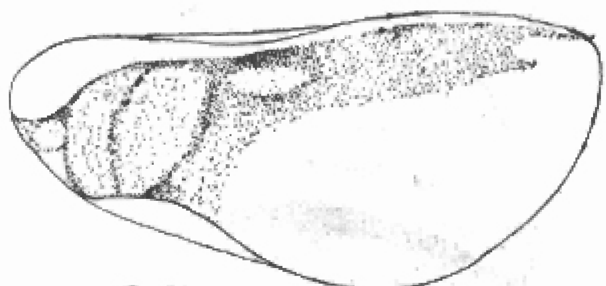
Fig27 St.16

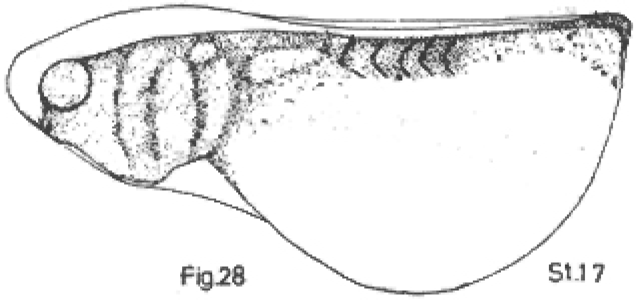

Fig.28 St.17

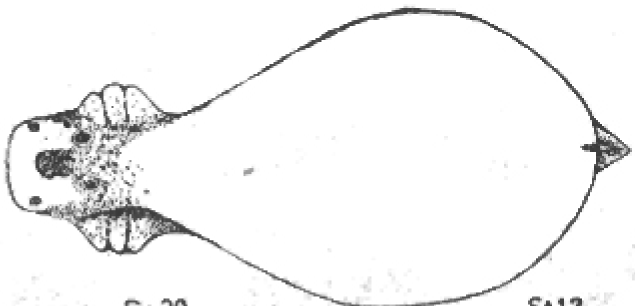

Fig.29 St.17

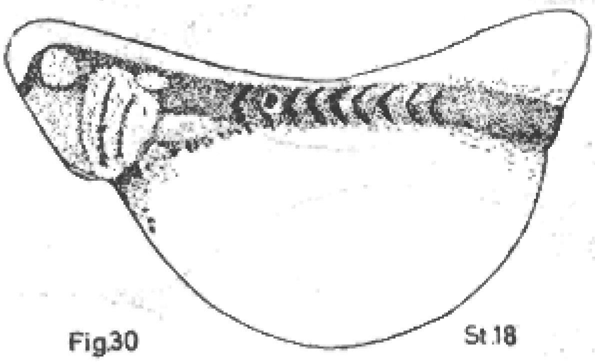

Fig.30 St.18

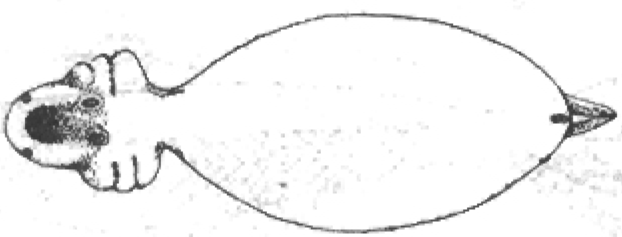

Fig.31 V.V. St.18

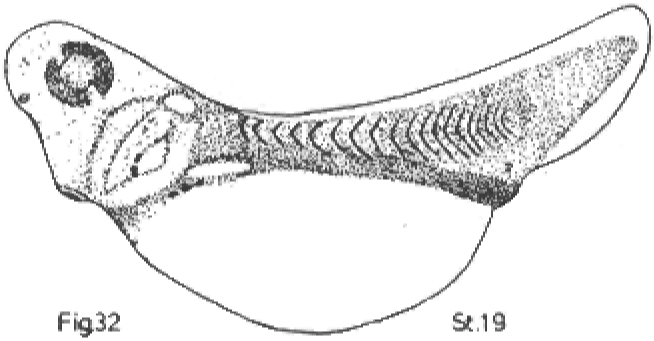

Fig.32 St.19

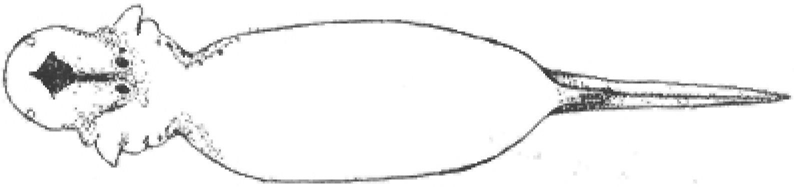

Fig.33 St.19

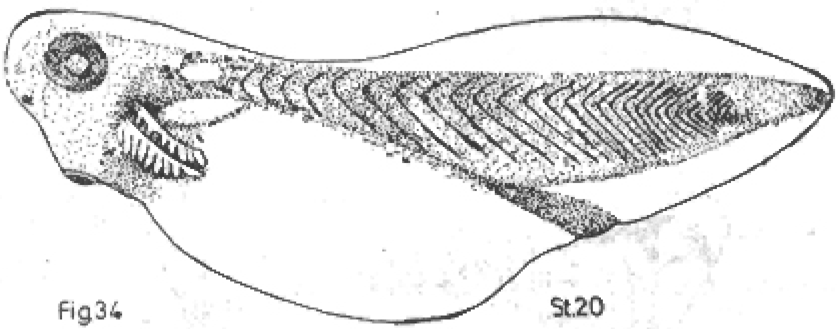

Fig.34 St.20

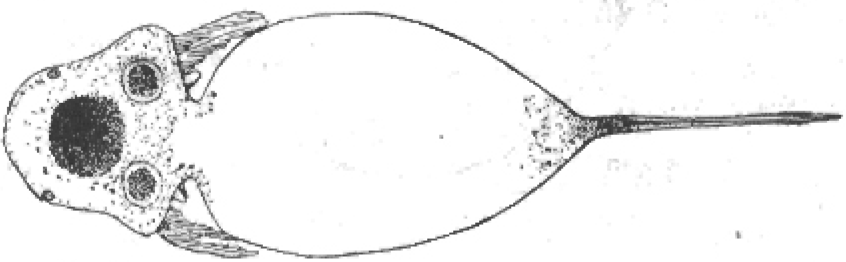

Fig.35 V.V. St.20

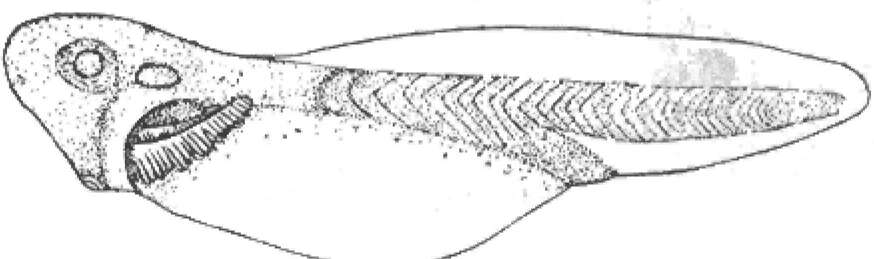

Fig 36 St.21

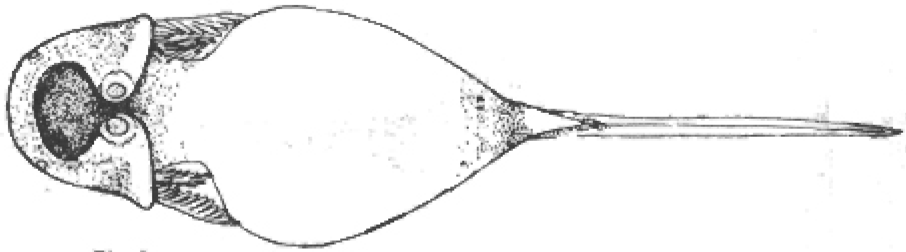

Fig. 37 St. 21

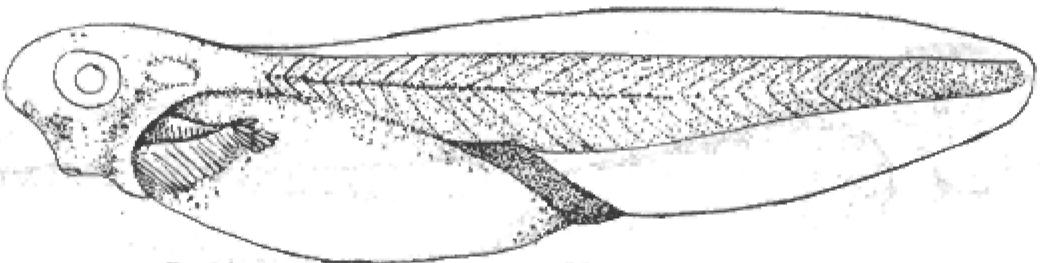

Fig 38 St 22

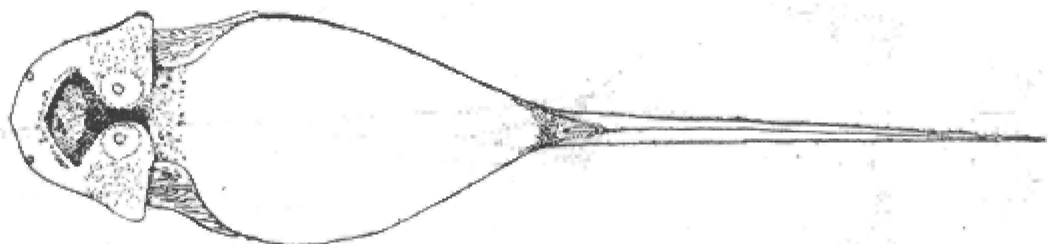

Fig.39 St.22

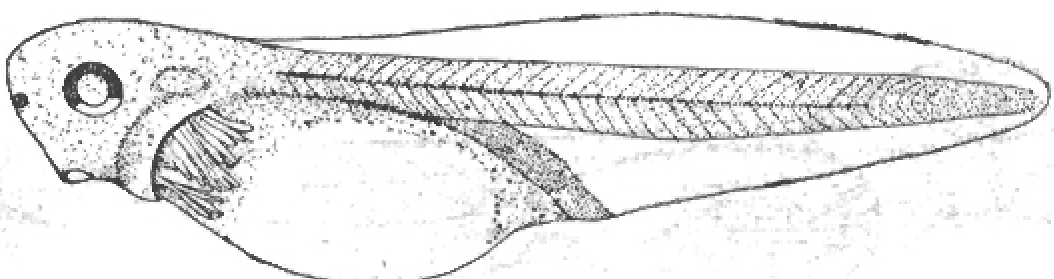

Fig40 St.23

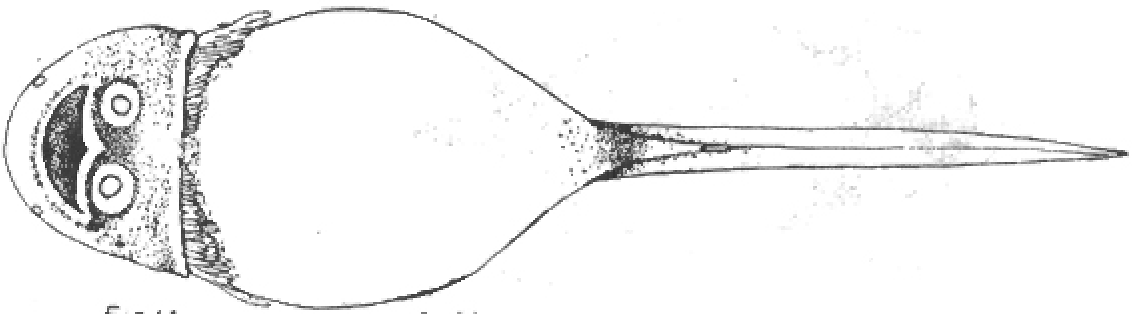

Fig.41 St 23

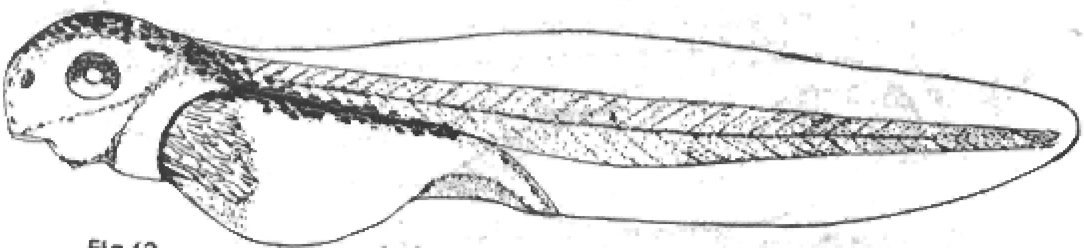

Fig.42 St. 24

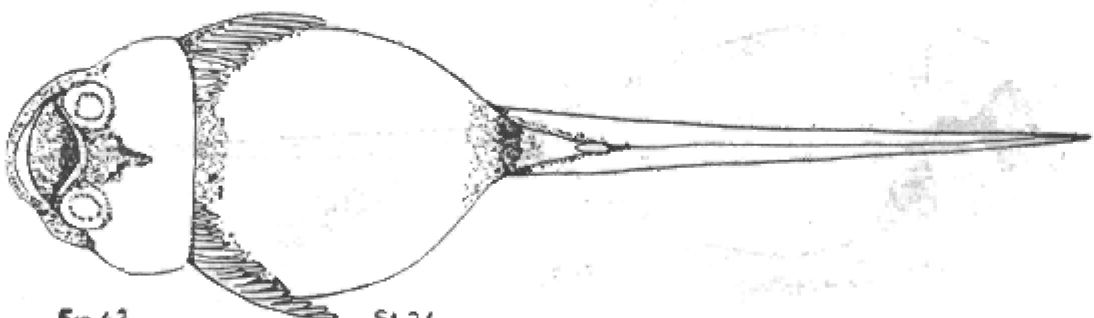

Fig.43 St.24

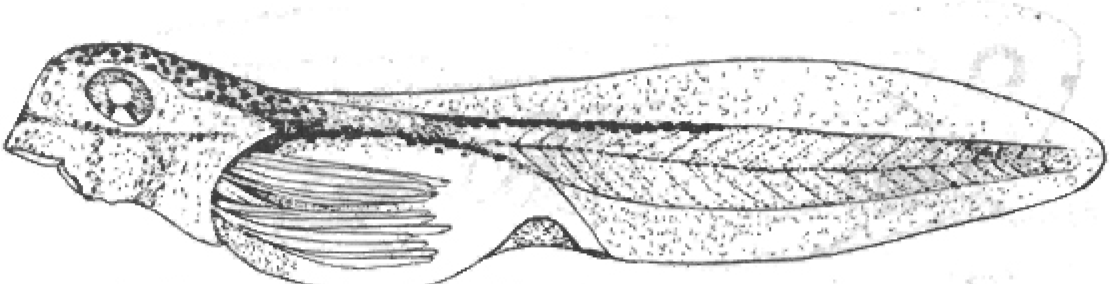

Fig.44 St.25

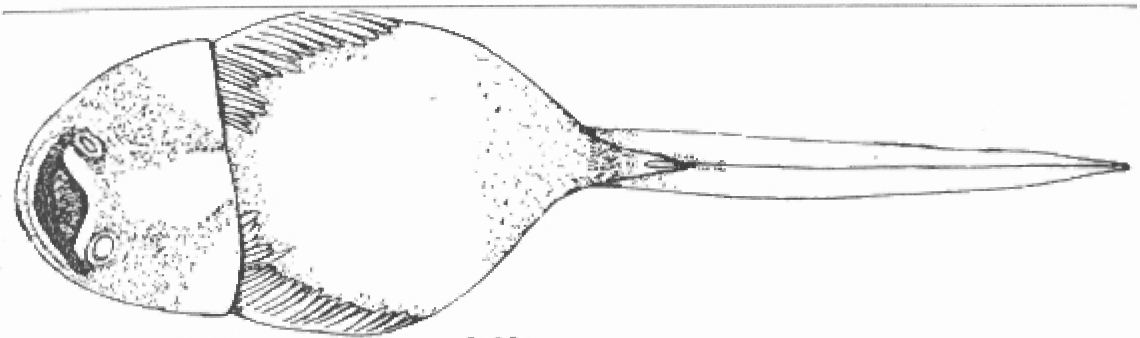

Fig.45 St.25

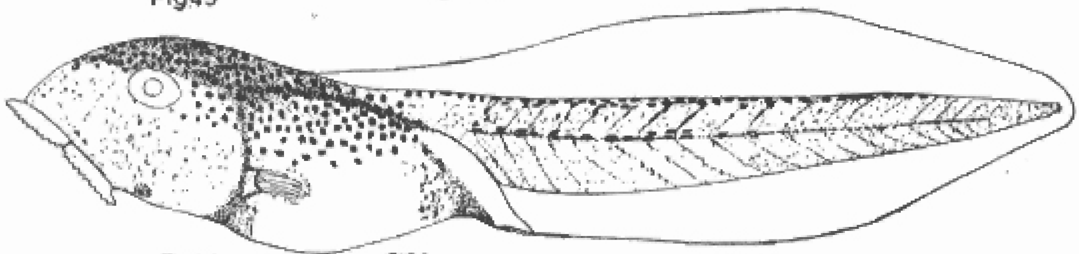

Fig.46 St.26

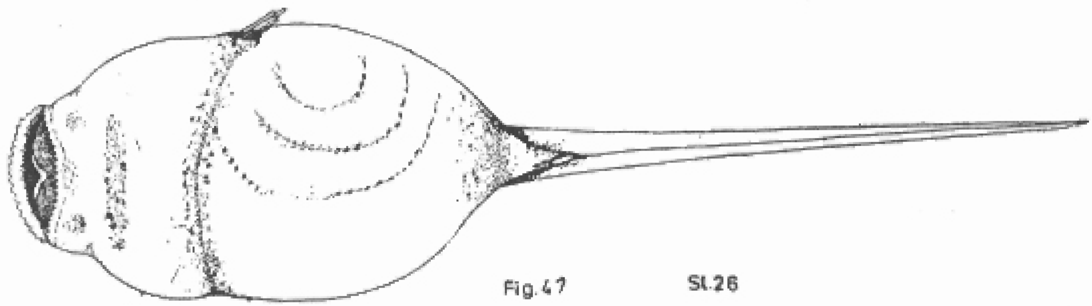

Fig.47 St.26

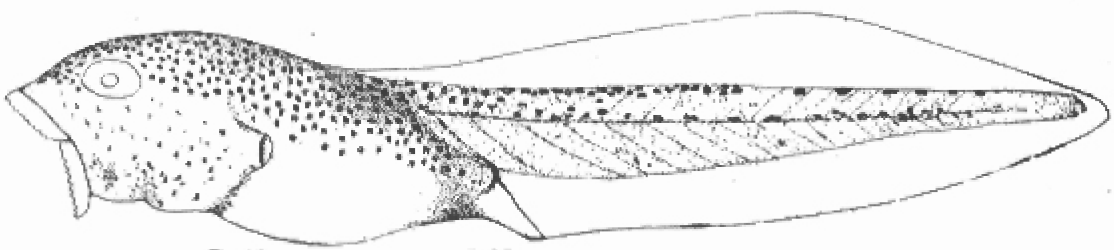

Fig.48 St.27

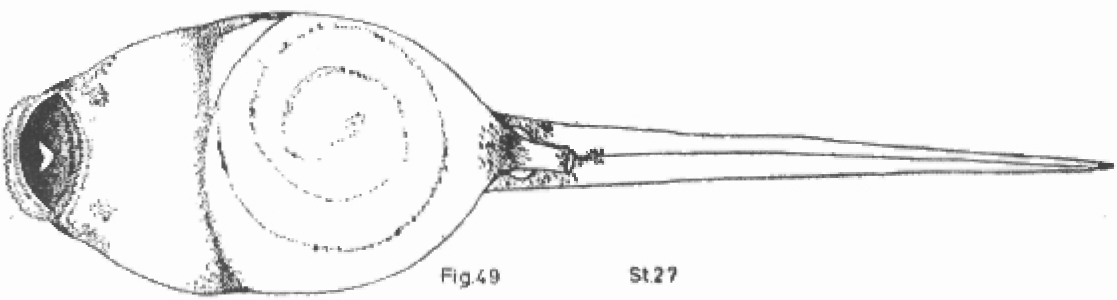

Fig.49 St.27

REFERNCES


1. Anderson. *Anat. Rec.* **86**: 58 (1943).
2. Balinsky, B. I. *An Introduction to Embryology.* W. B. Saunders and Co., Philadelphia (1966).
3. Cambar, R. & B. R. Marrot. *Bull Biol.*, 88, pp. 168-177 (1954)
4. Cambar, R. & M. S. Martin. *Act. Soc. Linn. Bord.* Vol. 98, pp. 1-20 (1956).
5. Cambar, & Gipouloux. *Bull. Biol.* 90, pp. 198-217 (1956).
6. Dan, K. *Inter. Rev. Cyto.* IX pp. 321-367 (1960).
7. Dean. *J. Morphol.* II : 1 (1895).
8. Del Conte, E. & Sirlin, J. L. *Anat. Rec* 125-135 (1952).
9. Eakin, R. M. *Univ. Calif. Pub. Zool.* 51, 245 (1946).
10. Eakin, R. M. *Univ. Calif. Pub. Zool.* 51, 245 (1947).
11. Eycleshymer, A. C. & J. M. Wilson *Entwicklungagschichte der Wirbeltiere*, Part II, F. Keibel. Gustab Fischer, Jena (1910).
12. Glasner, L. In F. Keibel, ed. *Normentalfelor G. Fisher, Jena.* V. 14 (1925).
13. Hamilton, H. L. & V. Hamburger. *J. Morph* 88, 49-92 (1951).
14. Harrison, R. G. (unpublished, 1955).
15. Jollie, M. "*Chordate Morphology*" Reinhold Pub., Co., N. Y. (1962).
16. Kerr, J. G. *Entwicklungagschichte der Wibeltiere*, Part 10. F. *Keibel. Gustab. Fischer*, Jena (1909).
17. Khan, M. S. *Biologia*, Vol. II, No. 1. pp. 1-39 (1965).
18. Kopch, F. (dargestallt in der Art der Normmentafeln Zur Entwicklungagschichte der Wirbelthiere) Stuttgard (1952).
19. Leavitt, N. in Rugh's *Experimental Embryology*, Burgess pub., Co., Minnapolis (1962).
20. Lillie, F. *Development of chick*, Holt Rinehart & Winston Inc., N. Y. (1952).
21. Linbauger, B. A. & E. P. Volpe *Am. Museum Novitates*, 1842 (1957).
22. Miller, D. C. *Proc. Indiana Acad. Sci.* 49, 209-214 (1939).
23. Nasir-ud-Din (unpublished) *M.Sc. Research* Panjab Univ. Dept. Zool (1965).
24. Nelson, E. O. *Comparative Embryology of Vertebrates*, the Blackistan Co. Ltd. N. Y. (1953).
25. Nieuwkoop & Faber *Normal Table of Xenopus laevis*. Daudin, North Holland Pub. Co., Amsterdam (1956).
26. Okada, Y. & K. Baba. *Frog Embryology* Iwanami, Tokyo (1932).
27. Oppenheimer. *Jour. Exp. Zool.* **73** : 405 (1936)
28. Outran. *Turtox News*, Vol. 37. No. 1, pp. 16-22 (1957).
29. Pollister, A. W. & J. A. Moore. *Anat. Rec.* 68, 489 (1937).
30. Rossi, A. *Monitare zool. Ital.* 66, 133—145 (1958).
31. Rugh, R. *Experimental Embryology*, Burgess Pub. Co. Minnapolis (1962).
32. Sedra, S. N. & M. I. Michael. *Morphologie*, Ix. 4. pp. 333-351 (1961).
33. Shumway. *Anat. Rec.* 78. 139-147 (1940).
34. Sirakami. *Mem. Fac. Liberal Arts Educ.* Yamanashi Univ. No. 9, 182 (1958b)
35. Solberg. *Jour. Exp Zool.* **78**: 445 (1958).
36. Tahara, Y. *Jap. Jour. Exp. Morph.* **13**: 49-60 (1959).
37. Tavolga, W. & R. Rugh. *Zoologica*, **32** : 1 (1947).
38. Taylor, A. C. & J. J. Kollros *Anat Rec.* 94, 7.23 (1946).
39. Twitty & Bodenstin in Rugh's *Experimental Embryology*, Burgess Pub. Co. Minnapoli (1965).
40. Weisz P. B. *Anat. Rec.* 93, 161-169 (1945).
41. Witschi, E. *XIV e Inter. Zool. Cong.* Copenhagen, August 1953 (1953).
42. Witschi, E. *Development of vertebrates* W. B. Saunders, Philadel. (1956).
43. Witschi, E. in *Hand Book of Biological Data*: Growth, Philadel. (1962b)